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Expression and Isolation of the BchE Encoded Protein of *Rhodobacter capsulatus in Rhodobacter capsulatus*

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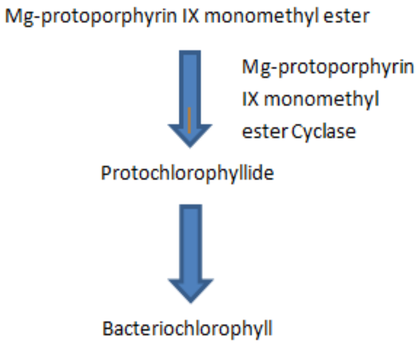
Introduction

Bacteriochlorophylls are light harvesting pigments that play an essential role in the process of photosynthesis in photosynthetic bacteria. Several of the enzymes involved in the synthesis of the tetrapyrrole bacteriochlorophyll *a,* are yet to be entirely understood. The step in which the ring structure of the tetrapyrrole is formed is catalyzed by the enzyme MPE-cyclase which converts the substrate MPE into Pchlide.



The gene *bchE* has been suggested to encode a protein required for MPE-cyclase activity in the photosynthetic bacterium *Rhodobacter capsulatus.* In order to study the cyclase enzyme, we attempted to isolate the polypeptide encoded by *bchE* by expressing the protein at increased levels using pRho expression vectors in *R. capsulatus.*

The vector pRhoks2:Rc BchE was mated into the *R. capsulatus* strain B10S (wild-type). The vector contains the *bchE* gene translationally fused to a StrepII Tag controlled by a kan cassette promoter. The vector pRhots2:Rc BchE was mated into B10S-T7 strain. This vector contains the *bchE* gene translationally fused to a StrepII Tag controlled by a fructose inducible promoter, pT7.

The goal of this project was to purify the Strep II tagged BchE protein for further studies and co-purify any strongly associated partners.

Materials and Methods

1. Growing Cells:
Anaerobic cultures for both strains of *R. capsulatus* were grown under light in RCV media for 48 hours. The B10S-T7 strain was induced with 1.2M fructose and both cultures were brought up to 300mL with RCV media.

2.Test for Protein Solubility:
Cultures were harvested with low speed centrifugation (12,000 rpm, 10 min @ 4°C) and lysed with Bugbuster Reagent (5 mL/g cells) containing benzonase (1uL/mL of BugBuster). The lysed cells were fractioned into an insoluble pellet (inclusion bodies, membranes, organelles, etc.) and soluble supernatant (cytoplasm, intracellular membranes, soluble proteins, etc.) via centrifugation at 16,000rpm for 20min @ 4°C. Proteins in the fractions were separated by SDS-PAGE and stained with GelCode Blue staining reagent

2. *Confirmation of BchE presence*:
A Western Blot of the SDS-PAGE protein gel was performed with a Novagen Kit to ensure the presence of BchE in the fractions. Protein was transferred electrophoretically to PVDF membrane and visualized with StrepII Tag monoclonal antibody HRP conjugate.

3. *Column Chromatography Purification*: Streptactin resin was used to purify BchE polypeptide with the StrepII Tag. The B10S-T7 soluble fraction was separated over the column. BchE was eluted off the column with 2.5mM Desthiobiotin solution which binds to streptactin with a higher affinity than StrepTag II. There were 6 (250 μL) elution fractions gathered. SDS-PAGE analysis was run on the 6 elution fractions.

Results

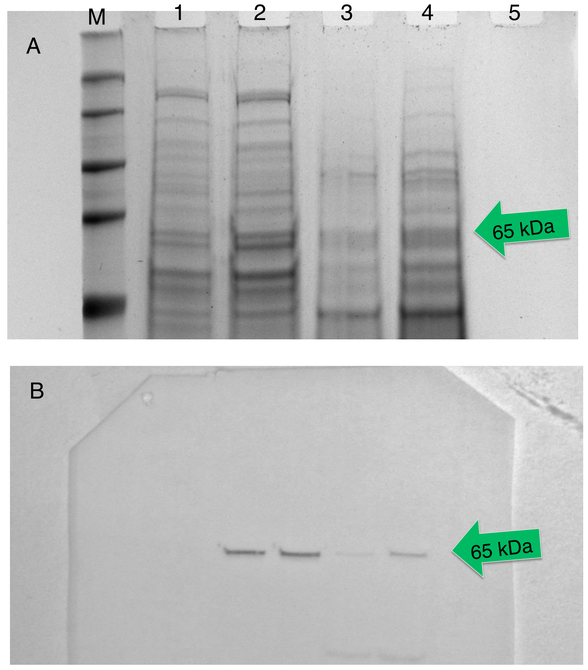


Figure 1. SDS-PAGE and Western Blot analysis of fractions from B10S and B10S-T7 strains. A. An SDS-PAGE analysis was prepared with 30μL of insoluble fraction for B10S and B10S-T7 and soluble fractions of B10S and B10S-T7 for lanes 1-4 respectively.
B. BchE polypeptide was visualized with StrepII Tag monoclonal antibody HRP conjugate. This confirmed the presence of BchE in the soluble fractions at 65kDa. M= marker.

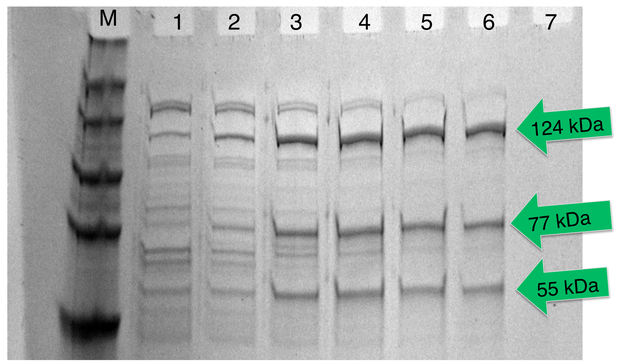


Figure 2. SDS-PAGE protein gel run from six 250μL elutions of soluble B10S –T7 fraction from a Strep-Tactin Column Chromatography gravity pull down. Each lane contained 40μL of the elution fractions. Mass spectrometry analysis of the gel revealed that the three distinct bands in lanes 3-6 were Pyruvate carboxylase (124kDa) and propionyl-CoA carboxylase subunits alpha (77kDa) and beta (55kDa). M= marker.

Conclusions

The MSMS analysis of the elution fractions from the chromatography column revealed that BchE was not purified. Pyruvate carboxylase was purified along with the two propionyl-CoA carboxylase subunits alpha and beta. These results indicated that biotin from the RCV media had out-competed the StrepII-tag of BchE for binding to the streptactin column thus leading to the purification of biotin utilizing enzymes. However, this confirmed that it is possible to co-purify protein partners with strong binding affinities.

Future Work

- In order to minimize the interaction between biotin and Streptactin, Avidin can be added to the soluble fraction of cultures which will bind biotin with a high affinity.
- Since BchE might be degrading or precipitating, future steps will be taken to minimize denaturing conditions. This includes using fresh samples, as opposed to freezing and thawing, and lysing the cultures via French Press.
- Protein associations with the membrane can be reduced by suspending pellets in salt solutions with detergent.

Literature Cited

Bollivar, David. "Intermediate Steps in Chlorophyll Biosynthesis: Methylation and Cyclization." *Chlorophylls and Bilins: Biosynthesis, Synthesis and Degradation.* 13. (2003): 49-68. Print.

N. Katzke et al. "A novel T7 RNA polymerase dependent expression system for high-level protein production in the phototrophic bacterium *Rhodobacter capsulatus.*" *Protein Expression and Purification.* 69 (2010) 137-146. Print.